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Department of General Microbiology, University of Copenhagen, Copenhagen, Denmark

Functional groups of microfungi on decomposing ash litter

ANNELISE KJØLLER*) and STEN STRUWE

With one figure

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1. Introduction

As a consequence of the long tradition for decomposition studies, different theories of succession and population structure have been proposed and are recently summarized in review-papers and books (e.g. SWIFT, HEAL & ANDERSON 1979, COLE & KENDRICK 1981, WICKLOW & CARROLL 1981, FRANKLAND, HEDGER & SWIFT 1982). The present paper presents a view of fungal decomposition of litter based on recent discussions of succession by PARK 1976, SWIFT 1976, 1982, 1984, FRANKLAND, 1981, 1982, and on organization of populations and strategies of fungi by PUGH 1980, WICKLOW 1981, KJØLLER & STRUWE 1982, HEAL & INESON 1984, and STRUWE & KJØLLER, 1986.

In our previous contribution on alder litter (*Alnus glutinosa* GÄRTN.), the role of fungal and bacterial decomposition was investigated (KJØLLER & STRUWE 1980, KJØLLER, STRUWE & VESTBERG 1985). We found indications of a high potential activity in the cold winter period, with a cellulolytic maximum in February. A *Phoma* sp. was the dominating cellulolytic fungus, other frequent fungi were *Cladosporium herbarum* LINK, ex FR. and *C. cladosporioides* (FRES.) DE VRIES, both with versatile activity patterns.

In the present study, ash litter (*Fraxinus excelsior* L.) and upper soil layers were examined monthly for two years. All samples were collected from an ash stand with high moisture and nitrogen contents, but without the supply of nitrogen compounds caused by the nitrogen fixing nodules found on the alder roots. Also the role of different functional groups of bacteria have been investigated in ash litter (STRUWE & KJØLLER 1985).

2. Material and methods

The site investigated was the same stand of ash (*Fraxinus excelsior*) as described in STRUWE & KJØLLER (1985). It is situated in a nature reserve, Strødam, North Zealand, Denmark.

The ash leaves were shed in November, and the site was waterlogged in the winter months. Samples of litter and the upper 5 cm of the organic horizon were collected each month during 2 years (1981–1983), the leaves only from litter fall in November until disappearance in May. The samples were processed the same day as collected.

Fungi were counted by the plate count technique. 10 g of fresh litter or subsoil was mixed in 990 ml Winogradsky's salt solution diluted 1: 20, WINOGRADSKY 1949, and blended in a Braun blender for 4 minutes. Decimal dilutions were also prepared in Winogradsky's salt solution. The number of yeasts and other fungi were counted on V-8 agar (V-8 juice, 200 ml; $CaCO_3$, 3 g; agar, 20 g; and distilled water, 800 ml; with addition of penicillin, 0.1 g; and streptomycin, 50 mg, after autoclaving.

All plate counts were made in 5 replicates, and the counts were estimated from suspensions of the same dilution. Fungi were isolated by the soil washing technique. Samples of fresh leaves or soil, approx. 10 g were blended with sterile Winogradsky's salt solution 1: 20 for 4–5 min. The mixture was washed through sieves of decreasing mesh size, and particles >0.4 mm were collected and washed in running tap water for 5 min, previously found to ensure absence of conidia. Using sterile forceps, single particles of leaf or soil were placed on V-8 agar and (Nov., Feb., May) also on soil extract agar diluted 1: 100 (glucose, 1.0 g; peptone, 1.0 g; yeast extract, 1.0 g; K_2HPO_4, 1.0 g; soil extract, 400 ml; dist. water, 600 ml; agar (Noble), 20 g).

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*) Corresponding author

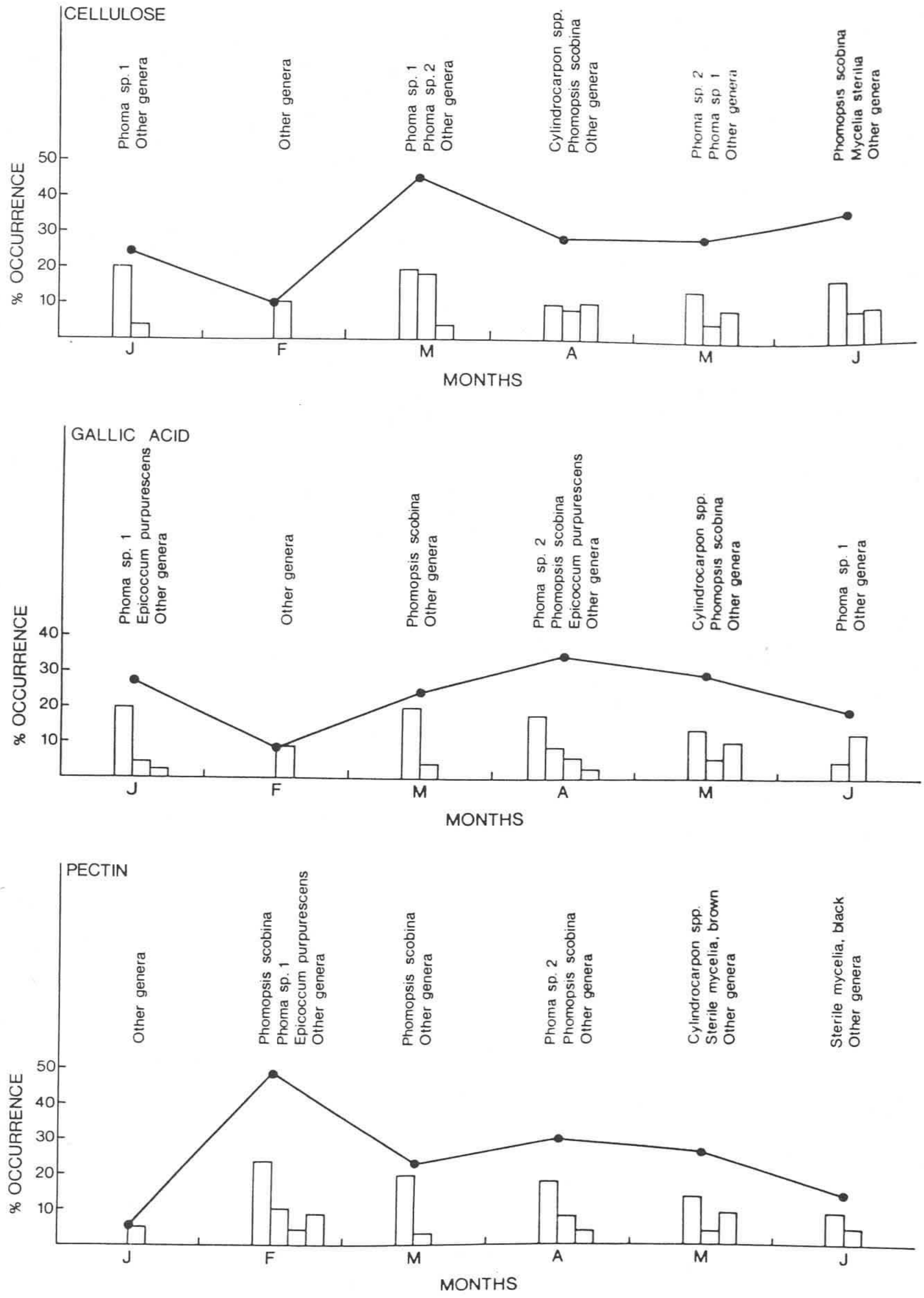
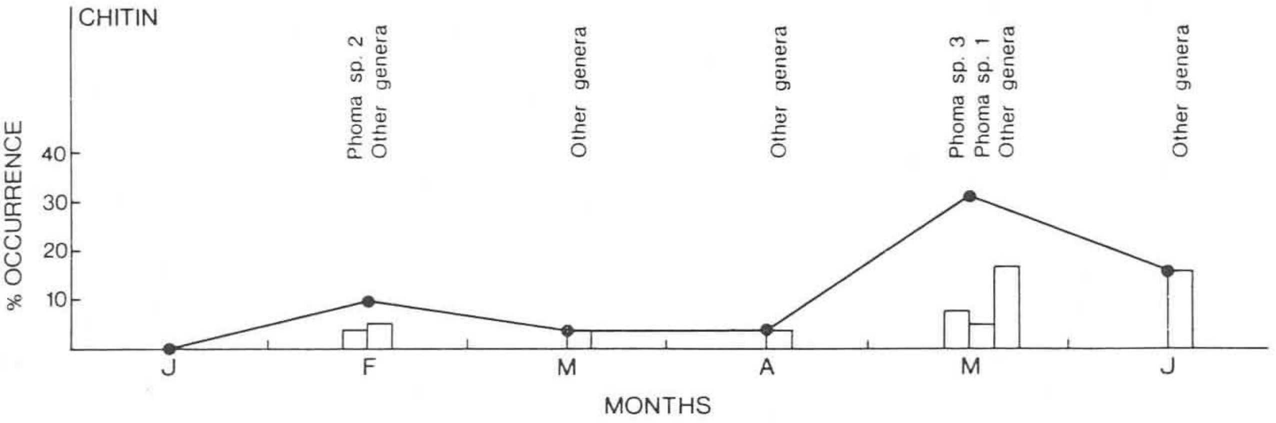
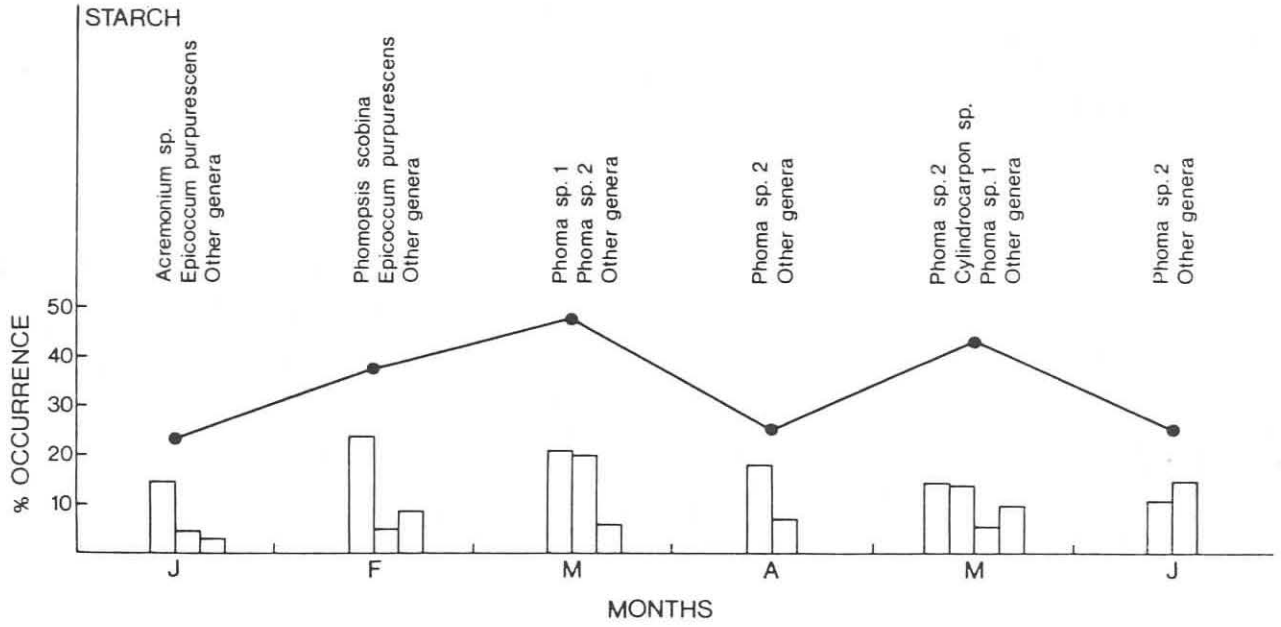
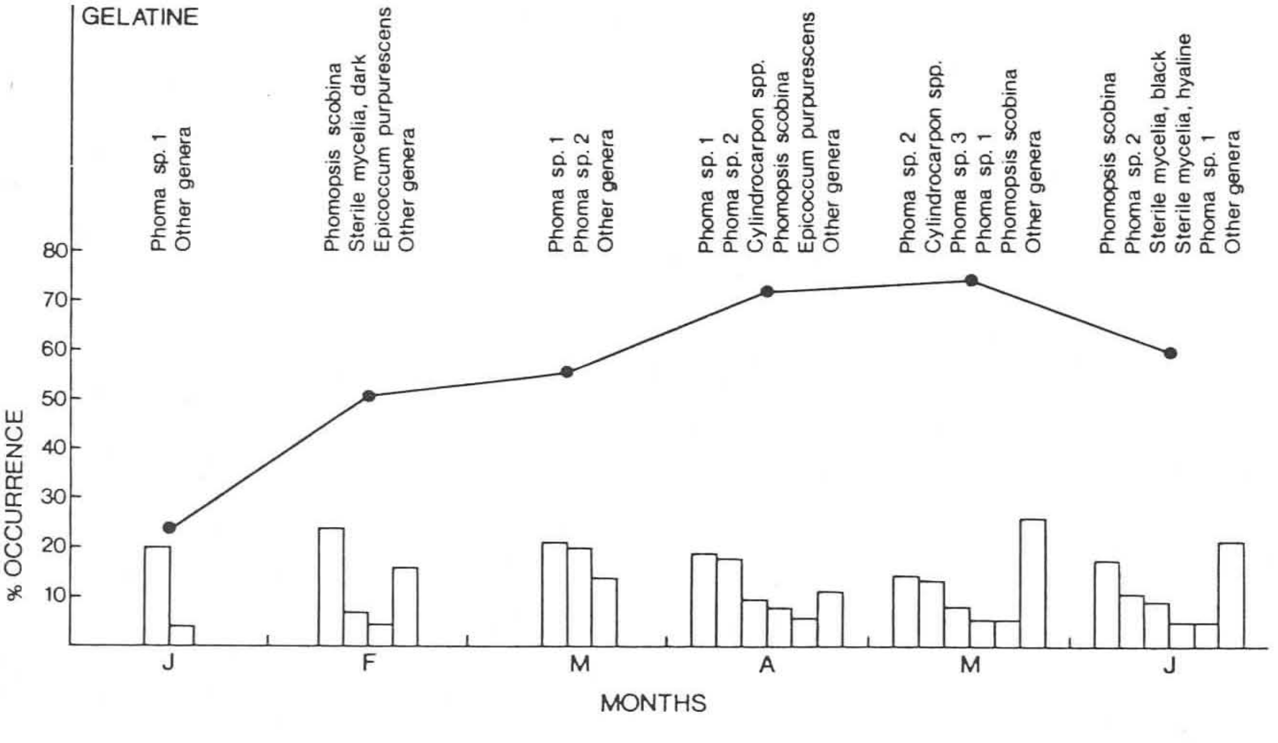


Fig. 1. The utilization pattern during decomposition of ash litter. The columns indicate the occurrence of the most frequent fungi on each litter compound. The graphs show the sum of the columns for each month, indicating the percentage of fungi able to utilize each litter compound.


CHITIN
% OCCURRENCE
10
20
30
40
Phoma sp. 2
Other genera
Other genera
Other genera
Phoma sp. 3
Phoma sp. 1
Other genera
Other genera
J
F
M
A
M
J
MONTHS


STARCH
% OCCURRENCE
10
20
30
40
50
Acremonium sp.
Epicoccum purpurescens
Other genera
Phomopsis scobina
Epicoccum purpurescens
Other genera
Phoma sp. 1
Phoma sp. 2
Other genera
Phoma sp. 2
Other genera
Phoma sp. 2
Cylindrocarpon sp.
Phoma sp. 1
Other genera
Phoma sp. 2
Other genera
J
F
M
A
M
J
MONTHS


GELATINE
% OCCURRENCE
10
20
30
40
50
60
70
80
Phoma sp. 1
Other genera
Phomopsis scobina
Sterile mycelia, dark
Epicoccum purpurescens
Other genera
Phoma sp. 1
Phoma sp. 2
Other genera
Phoma sp. 1
Phoma sp. 2
Cylindrocarpon spp.
Phomopsis scobina
Epicoccum purpurescens
Other genera
Phoma sp. 2
Cylindrocarpon spp.
Phoma sp. 3
Phoma sp. 1
Phomopsis scobina
Other genera
Phomopsis scobina
Phoma sp. 2
Sterile mycelia, black
Sterile mycelia, hyaline
Phoma sp. 1
Other genera
J
F
M
A
M
J
MONTHS

The isolates were identified to generic level and tested for utilisation of gelatine, starch, pectin, cellulose, chitin and gallic acid as in the previous study of alder litter (KJØLLER & STRUWE 1980). The isolates from diluted media were tested only on gallic acid. All cultures were incubated at 10 °C and the incubation time varied from one week to one month.

3. Results

The numbers of colony forming units (CFU) of microfungi and yeasts are shown in Table 1. The numbers of fungi were slightly increased during decomposition of the leaves, from 0.9×10^7 per g of dry litter, in December to 3.8×10^7 in April. In the organic layer the number was highest in the winter months approx. 2.5×10^6 per g of dry material, lower in the summer, approx. 1.3×10^6. The numbers of yeasts were high at the beginning and at the end of the decomposition period, $< 10^8$ per g of dry litter, lower during the winter, approx. 2.5×10^7. In the organic layer the numbers of yeasts were also low in the winter, lower than 10^6 per g of dry material and higher in summer and autumn reaching 6.4×10^6 in August.

The soil washing technique showed that the colonization ratio (number of isolates per number of particles) GAMS & DOMSCH (1967) was higher in litter than in the organic layer. The ratio ranged from 1.2—1.7 in litter and with values around 1 in the upper organic horizon. Between 15 and 37 different taxa were identified from the litter, and from 21 to 42 in the organic layer. The same colonization ratio was reached on the diluted medium, but the fungal population was composed of a limited number of taxa.

Tab. 1. CFU of microfungi and yeasts in litter ($\times 10^7$) and organic horizon ($\times 10^6$) per g of dry matter. (The litter was decomposed in June)

	Fungi		Yeasts	
	Litter	Organic horizon	Litter	Organic horizon
Nov.	2.6	1.4	1.7	1.5
Dec.	0.9	1.4	0.9	0.4
Jan.	1.9	2.7	4.0	0.2
Feb.	2.8	2.3	1.3	0.1
Mar.	2.7	1.1	2.2	0.7
Apr.	3.8	0.5	0.9	nd
May	2.4	0.9	1.2	0.3
June	—	1.1	—	1.1
July	—	nd	—	nd
Aug.	—	1.8	—	6.4
Sept.	—	0.7	—	1.3
Oct.	—	0.6	—	0.5

Tab. 2. The number of isolates of the most frequent fungi during decomposition of ash litter expressed as a percentage of the total number of isolates in each monthly sample*)

	Relative (%) occurrence					
	J	F	M	A	M	J
Phomopsis scobina	29.5	30.6	20.0	8.1	5.7	17.8
Phoma sp. 1	19.9	13.5	21.3	19.3	5.7	5.1
Acremonium sp.	14.5					
Mycelia sterilia, grey		9.9				
Epicoccum purpurescens		6.3		6.0		
Phoma sp. 2			20.0	17.8	14.6	10.2
Cylindrocarpon sp.				9.6	13.8	0.8
Mycelia sterilia, black						10.2
Mycelia sterilia, hyaline						9.3
Sphaeropsidales						4.2
% of Total	63.9	60.3	61.3	64.8	47.9	52.5

*) The fungi were not isolated in the months without figures.

Table 3. The number of isolates utilizing each of the six pure substrates expressed as a percentage of the total number of isolates in each sample from the organic horizon during one year

	Cellulose	Gallic acid	Pectin	Chitin	Starch	Gelatine
May	7.2	29.1	21.8	16.5	17.3	39.3
June	2.1	26.9	34.4	11.3	20.6	32.1
July	13.2	32.0	22.0	7.7	39.8	31.0
Aug.	15.4	44.1	13.7	5.3	10.1	52.0
Sep.	6.6	6.1	30.5	nd.	22.6	42.2
Okt.	25.3	18.6	18.2	13.2	13.7	42.0
Dec.	1.2	29.6	2.4	27.2	38.1	38.9
Jan.	38.8	21.4	31.0	18.5	28.3	32.2
Feb.	11.0	16.0	1.0	36.0	7.0	21.0
Mar.	21.2	28.0	29.1	26.8	11.1	20.1

The data from the fungal utilization of six pure compounds are shown in Fig. 1 and Tab. 3. and the most frequent fungi during decomposition in Tab. 2.

The results below refer to the 1982—1983 figures. The figures from the two years were very similar, but the cellulose test was missing from 1981—1982.

The frequency of fungi able to utilize gelatine was low during the winter months (approx. 20%) in the organic layer. and between 31 and 52% of the isolates in spring and summer. In the litter the number of proteolytic fungi increased towards the end of the decomposition period from approx. 25% in January to 75% in May.

Many different fungi were able to utilize gelatine, and important genera in the organic layer were *Mortierella* spp.. *Phoma* spp., *Fusarium* spp., *Trichoderma viride* PERS. ex. FR. and sterile mycelia. In the litter, *Phoma* spp. and *Phomopsis scobina* HÖHN. were dominant gelatine utilizers, together with *Cylindrocarpon* spp., *Epicoccum purpurescens* EHRENB. et SCHLECHT. and sterile mycelia.

Amylolytic fungi were present in all samples of litter and the organic horizon during the year, with highest occurrence during the winter months December and January and in July. consisting of approx. 30—40% of the isolates. In the litter 15—45% of the isolates showed amylolytic activity.

A number of different fungi showed amylolytic activity. In the organic layer the active fungi were *Phoma* spp., *Mortierella* spp., *Fusarium* spp. and sterile mycelia. In the litter, *Phomopsis scobina*, *Epicoccum purpurescens*, *Phoma spp. Cylindrocarpon spp.* and sterile mycelia were amylolytic.

The frequency of fungi with pectinase activity fluctuated in both the organic and the litter layer, ranging from low percentages to 34% of the isolates in the organic layer, and from 5% to 50% in the litter. The highest pectinolytic activity was observed in the winter months in both the organic layer and litter.

A variety of fungi were able to utilize pectin, in the organic layer: *Mortierella* spp.. *Phoma* spp.. *Fusarium* spp.. *Trichoderma viride* and sterile mycelia. and in the litter: *Phomopsis scobina*, *Phoma* spp.. *Cylindrocarpon* spp. and sterile mycelia were the most frequent pectinolytic isolates The occurrence of cellulolytic fungi isolated from the organic layer varied from 1 to 8%. In the litter the peak activity of cellulolytic fungi occurred in March when 45% of the isolates showed cellulolytic activity. Cellulolytic fungi in the organic layer were mainly species of *Trichoderma*, *Mortierella* and *Fusarium* and *Myrothecium verrucaria* (ALBERTINI *et* SCHWEINITZ) DITMAR. Other fungi were also responsible for the cellulose decomposition in the litter e.g. *Phoma* spp., *Cylindrocarpon* spp.. *Phomopsis scobina* and *Epicoccum purpurescens*.

The frequency of chitinolytic fungi in the organic layer fluctuated; during the year of investigation, 5—36% of the isolates were chitinolytic. In the litter the most frequent occurrence of chitinolytic fungi 10—30% was in December, April and May.

The most dominating chitinolytic fungi in the organic layer during the winter months were different unidentified species of *Mortierella*, in the summer months other fungi accompanied *Mortierella*, e.g. sterile mycelia *Cylindrocarpon* and *Diheterospora*. No particular fungus was responsible for the chitinolytic activity in the litter, among the most frequent were *Phoma* spp. and sterile mycelia.

Lignin decomposition, as indicated by gallic acid oxidation, took place in the organic layer at all seasons. Only two samples showed low potential activity, in the remaining, between 15 and 65% of the isolates were lignin decomposers. In the litter the activity was high apart from the coldest winter month February and when the litter was almost decomposed in May and June.

Fungi responsible for lignin decomposition in the organic layer were species of *Mortierella*, *Fusarium*, *Phoma* and sterile mycelia, all known to oxidize gallic acid, except for *Mortierella* (DOMSCH, GAMS & ANDERSON 1980). In the litter, sterile mycelia and *Cylindrocarpon* spp. were also frequent lignin decomposers, but furthermore, *Phomopsis scobina* and *Phoma* spp. also oxidized gallic acid.

On the diluted soil extract media *Mortierella* spp., *Acremonium* spp., *Penicillium* spp. and sterile mycelia were the most frequently isolated fungi from both litter and the organic horizon, furthermore *Alternaria alternata* (FR.) KEISSL. was found only in litter samples and *Myrothecium verrucaria* only in the organic layer. The most frequent isolates on the complex media, *Phoma* spp. and *Phomopsis scobina*, were not recorded on nutrient-poor media. The test on gallic acid showed approx. 25% of the isolates with ligninolytic activity in the litter in November and February and approx. 6% in May in the organic horizon.

4. Discussion

The ability of fungi to utilize pure litter components is used to establish a decomposition pattern. The first investigations of simultaneous changes in fungal populations and their potential role of litter decomposing ability were made by FLANAGAN & SCARBOROUGH (1974) in their tundra studies. Fungal decomposition of ash litter was previously investigated by HERING (1965) and FRANKLAND (1975, 1982). In HERINGS investigation, he found *Phomopsis scobina* as the most frequently isolated fungus during the first months of decomposition. In the present investigation *P. scobina* was also the most frequently isolated species. *P. scobina* showed a versatile utilization pattern during the entire decomposition period, with a potential to degrade gelatine, pectin, gallic acid and starch, and occasionally cellulose. These abilities combined with a rapid linear growth rate, 6 cm in 2 weeks (HERING 1965), make *P. scobina* an important invader and decomposer of ash leaves. The frequency of *P. scobina* decreased during the decomposition period, and it was replaced by *Phoma* spp., *Cylindrocarpon* spp., *Epicoccum purpurescens* and other genera. In comparison with the fungal population of alder litter (KJØLLER & STRUWE 1981) the population on ash litter was differently composed and did not include common genera such as *Cladosporium*, *Alternaria* and *Aureobasidium* among the dominant fungi from January until June.

As the decomposition of ash litter proceeds, a change in dominant strains was seen. Initially very few fungi constitute the dominating population, but towards the end more genera were included. This resulted in an increased diversity, because the number of isolates varied only little. *Phomopsis scobina* and *Phoma* sp. 1 were the dominating genera from January to March but active through the entire period, in April and May another *Phoma* sp. 2 and *Cylindrocarpon* spp. were the most frequent. At the end of the decomposition period (June) the group of less frequently occurring fungi (other genera in Fig. 1) was of importance. It was characteristic that the major chitin degradation in the litter was carried out by fungi other than the *Mortierella* spp., which were the most active chitinolytic fungi in the organic layer. The most important cellulose decomposers were the different *Phoma* species, as in the alder litter (KJØLLER & STRUWE 1980).

When the litter was decomposed in June a marked shift in the composition of the fungal flora took place in the organic horizon. Here different species of *Mortierella* were the most frequently isolated fungi each month, in the summer approx. 40% of the isolates, in spring, autumn and winter approx. 25%, but their ability to decompose the different litter compounds (espc. chitin) varied from month to month. Other important fungi in the organic horizon were *Trichoderma* spp. and *Fusarium* spp., able to degrade cellulose, starch, gallic acid, and pectin, and *Cylindrocarpon* spp., chitin and gelatine. The isolated species of *Acremonium*, *Phoma*, *Rhinocladiella*, *Tetracladium* and *Diheterospora*, had different patterns of utilization. In the alder swamp the organic layer was only investigated 4 times during the year, so a direct comparison with this investigation is not possible. Also here *Cladosporium* spp. were the most frequent isolates together with species of *Phoma*, *Acremonium*, *Penicillium*, *Cylindrocarpon* and *Mortierella*.

The fungal potential for cellulose and chitin degradation in the upper organic layer of the ash stand was very low during the summer period. After litter fall in October these activities increased, reaching a peak for cellulose in March, and later for chitin in May. It was previously shown (STRUWE & KJØLLER 1985) that the number of bacteria utilizing chitin was high from December to April and decreased in May when the fungal potential for chitin-utilization was high. The gallic acid degrading population in the organic layer exhibited a utilization pattern which was the reverse of that of cellulose and chitin, i.e. when these compounds are used up in June, the potential lignin decomposers increased.

Nutrient-poor soil extract media were used to isolate slow growing, low nutrient demanding or oligotrophic fungi from litter and soil. Apart from the absence of *Phoma* and *Phomopsis*, the population was similar to the one on the usual nutrient-rich media. The test on gallic acid did not show a high percentage of ligninolytic activity among the isolated fungi, only approx. 25% at the beginning of decomposition of the litter and as few as 6% towards the end. In the underlying organic horizon also very few percentages — less than 6% — of the nutrient-poor isolates could utilize gallic acid.

DE BOOIS (1976) and REMACLE (1971) investigated oak litter decomposition in Holland and Belgium. DE BOOIS found *Aureobasidium* as the first cellulose decomposer, while later (after six months) *Mortierella* constituted 50% of the isolates. Other soil-inhabiting fungi such as *Trichoderma* and *Penicillium* also invaded the litter, but with a low decomposition rate. In his discussion of the succession of oak litter microflora, REMACLE states that it is not clear why the composition of the fungal population changes during decomposition, but is probably due to the "adaptive ability of the fungi".

Recently GRUNDA & MARVANOVA (1982) published results from an IBP study of microfungi in the soil layers of a seasonally flooded floodplain forest in Czechoslovakia *(Ulmus, Fraxinus, Quercus)*. The highest number of fungi was reached in the winter with a peak in February in the upper organic layer, and the average was 2.8×10^6 per g of dry matter. The figures are very similar to our data from the ash stand. Their numbers of cellulolytic and ligninolytic fungi constituted 2.2 and 3.2% of the total number respectively, increasing with depth to 6 and 23%. The enumeration method was different (plate count), so a direct comparison of frequencies is not meaningful. The fungal populations were quite similar and important ligninolytic fungi were *Cylindrocarpon*, *Penicillium*, *Sporothrix* and *Phoma*, and it is assumed that these fungi participate in the humus-forming process.

5. Conclusion

The examination of the populations of microorganisms and their function in the alder and ash litters are completed with this contribution. The fungal utilization potential is in such good agreement with the sequence in the decomposition of individual litter compounds (MIKOLA 1955), that we find it justified to use the potential as a measure of actual decomposition of the litter. The corresponding values for bacteria showed high bacterial numbers, but only little seasonal variation, mainly connected with nitrogen availability and moisture. This indirect evidence of the relative importance of fungi is supported by the respiration figures of forest litter and soil provided by ANDERSON & DOMSCH (1975).

It therefore seems a valid approach to combine data on the occurrence of organisms with their function as decomposers. Other strategies are necessary to examine decomposition rates of individual litter compounds or metabolic activity of populations, but as a consequence the detailed information on the organisms and their function is lost.

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Address of the authors: ANNELISE KJØLLER and STEN STRUWE, Afdeling for General Mikrobiologi, Københavns Universitet [Department of General Microbiology, The University of Copenhagen], Sølvgade 83H, DK - 1307 København K.

Synopsis: *Original scientific paper*

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The fungal population on decomposing litter and upper organic layer of an ash stand was isolated monthly by the soil washing technique.

The isolates were tested for their ability to utilize six pure litter compounds, gelatine, starch, pectin, cellulose, chitin and gallic acid. The highest potential of cellulose decomposition appeared in March caused by *Phoma* spp. The most frequent fungus in the litter was *Phomopsis scobina* with a versatile utilization pattern, and in the upper organic horizon, *Mortierella* spp. were able to utilize all the test compounds including gallic acid. A marked shift in the composition of the fungal flora was observed, when the litter was decomposed in June.

Key words: Soil fungi, decomposition, ash litter, gelatine-, starch-, pectin-, cellulose-, and chitin-utilization.